

Burden of *Aeromonas hydrophila* –associated diarrhea among children younger than 2 years in rural Egyptian community

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Abstract

Introduction: Between 2004 and 2007, a birth cohort of Egyptian children was analysed to evaluate the epidemiology of enteric diseases.

Methodology: A stool sample was collected from the study children every two weeks as well as whenever they experienced diarrhea. Samples were tested for routine bacterial pathogens as well as enteropathogenic viruses and parasites. A secondary goal of the study was to evaluate the burden of less commonly reported pathogens including *Aeromonas hydrophila*.

Results: Of the 348 study subjects, 79 had *A. hydrophila* isolated from their stool at some point during the study. Thirty-six children had exclusively symptomatic (S) infections while 33 had exclusively asymptomatic (AS) infections. However, 10 children had both S and AS infections. Among symptomatic cases, *A. hydrophila* was the sole pathogen isolated 36% of the time. An important aspect of *A. hydrophila* associated diarrhea was the high level of resistance to cephalosporins.

Conclusion: Although relatively uncommon, *A. hydrophila* was found to be associated with diarrhea among children living in Egypt and was frequently multi-drug resistant.

Key words: *Aeromonas hydrophila*; diarrhea; children

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Introduction

Diarrhea is a leading cause of childhood mortality and morbidity in developing countries and ranks among the most common causes of disease in children worldwide [1]. Among bacterial etiologies of diarrhea, *A. hydrophila* is recognized increasingly as a clinically significant enteric pathogen [2-4]. However, there are limited data on the prevalence and associated severity of diarrheal disease caused by *A. hydrophila* in many regions, including the Middle East and Africa [4]; furthermore data from the region were conflicting. Although in a study of children living in Cairo, Egypt, *Aeromonas* spp. was commonly isolated from diarrheal stools [5], the organism was rarely isolated from the diarrheal samples of children living in Alexandria, Egypt, during the same reporting period [6]. Thus the current study was designed to determine if *A. hydrophila* was associated with gastroenteritis in

children younger than two years of age and living in a rural community in Egypt.

Methodology

Stool samples were cultured aerobically on MacConkey (Becton Dickenson, Cockeysville, MD, USA) and *Salmonella-Shigella* (Becton Dickenson, Cockeysville, MD, USA), Skirrow's selective medium (Becton Dickenson, MD, USA) and thiosulfate-citrate-bile salts-sucrose (Becton Dickenson, MD, USA) agar plates and evaluated for the presence of *Shigella* spp, *Salmonella* spp, *Campylobacter* spp, *Vibrio cholerae*, Enterotoxigenic *Escherichia Coli* (ETEC) and *A. hydrophila*. Lactose-negative colonies were further characterized using a 4-tube biochemical procedure. Isolates testing positive for oxidase, indole and motility but negative for ornithine, urease and hydrogen sulfide were identified presumptively as *Aeromonas* species. All suspected colonies were

subsequently confirmed to be *A. hydrophila* using the API 20E system (BioMérieux SA, Marcy l'Etoile, France).

Antibiotic susceptibility to ampicillin (AMP, 10µg), cephalothin (CR, 30µg), streptomycin (STR, 10µg), chloramphenicol (CHL, 30µg), nalidixic acid (NA, 30µg), trimethoprim/sulfamethoxazole (SXT, 25µg) and ciprofloxacin (CIP, 5µg) was performed using the disk diffusion method according to the criteria recommended by the Clinical and Laboratory Standards Institute [7].

Additional stool testing

Commercially available enzyme immunoassay (EIA) diagnostic kits were used according to the manufacturer's instructions to test for the presence of rotavirus (Premier RotacloneR, Meridian Bioscience, Cincinnati, OH, USA), adenovirus, norovirus and astrovirus (IDEIA Oxoid (Ely) Ltd, Denmark House, Angel Drove, UK). Detection of *Cryptosporidium* and *Giardia lamblia* was performed using a commercially available EIA (*Cryptosporidium* II, *Giardia* II and *E. histolytica* II kit, Techlab, Blacksburg, VA, USA).

Statistical analysis

Statistical analysis was performed by the chi-square test. The differences were considered significant when the *P* value was less than 0.05. Crude relative rates were computed as a ratio of the incidence rates in the presence and absence of *A. hydrophila* infection; likelihood ratios were also calculated. Statistical analyses were performed with SAS software (version 9.1, SAS Institute Inc, Cary, NC, USA).

Definitions

Symptomatic (S): A child was considered symptomatic if he or she had at least one diarrheal day. A "diarrheal day" was defined as the occurrence of at least three non-formed stools (or at least one if bloody) in a 24-hour period. In addition, if the child was breastfed and the stool was not bloody, the mother had to report an increase in frequency or a reduction in consistency of the stools, compared with what she considered to be normal. The duration of a diarrhea episode was defined as beginning on the first diarrheal day after at least three consecutive non-diarrheal days and ending when followed by at least three consecutive non-diarrheal days.

Pathogens, including *A. hydrophila*, were considered to be related to a diarrheal episode if they were detected on any day of a diarrheal episode. An *A. hydrophila* episode was considered to have a co-

pathogen if *Shigella* spp, *Campylobacter* spp., *Salmonella* spp., ETEC, *Cryptosporidium*, adenovirus, norovirus, astrovirus or rotavirus was identified in a fecal specimen collected during the episode.

Asymptomatic (AS) child: A child without any loose or liquid stools for a period including the day of the visit and three days before or after the visit day was considered asymptomatic.

Results

Three-hundred forty-eight children were enrolled in the birth cohort. *A. hydrophila* was isolated from 1.4% (56/4001) of stool samples from children with diarrhea and from 0.5% (52/9539) of stool samples collected from children without diarrhea (AS). The 56 episodes of *A. hydrophila*-associated diarrhea occurred among 46 children with 6 subjects having 2 episodes and one subject having 5 episodes of *A. hydrophila*-associated diarrhea. *A. hydrophila* was isolated as the sole pathogen in 20 episodes (36%) (Figure 1). In AS samples, the 52 isolates of *A. hydrophila* were detected among 43 children and only 4% (n = 2) were detected as a sole organism. The relative risk of having diarrhea among children with *A. hydrophila* isolated in the stool compared with children without *A. hydrophila* isolated in the stools was 3.7 (95% confidence interval 2.7-3.5). The difference in isolation of *A. hydrophila* as a sole pathogen from S and AS samples was highly significant ($p < 0.001$), and the likelihood ratio for having diarrhea was 36.9 when *A. hydrophila* was isolated as a sole pathogen.

In 26 episodes, *A. hydrophila* was isolated with at least one other pathogen. The most common co-pathogens identified were ETEC (n = 14), *Campylobacter* spp. (n = 4), rotavirus (n = 6), and *Shigella* spp. (n = 3). In 10 stool samples, the possibility for a co-infection could not be excluded as the quantity of stool sampled was not sufficient to allow testing for adenovirus, norovirus, astrovirus or *Cryptosporidium*. For stool samples among AS children, *A. hydrophila* was isolated from the stool along with other pathogens from 48 stool samples. Among the most frequent microbes detected were ETEC as a sole infection (n = 31), ETEC and adenovirus (n = 2), and ETEC and *Campylobacter* spp. (n = 3). For two stool samples from AS children, the quantity of stool collected was insufficient for testing for adenovirus, norovirus, astrovirus or *Cryptosporidium*.

Figure 1. Isolation rates of *A. hydrophila* in symptomatic (S) and asymptomatic (AS) children

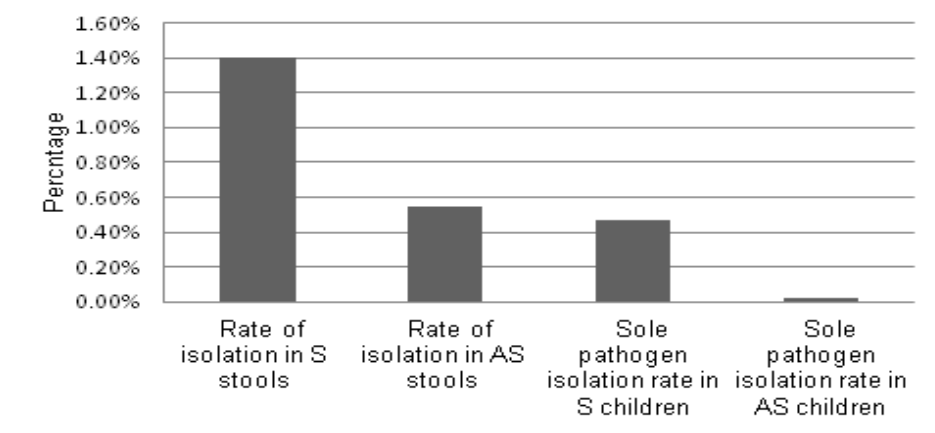


Figure 2. Resistance pattern of symptomatic and asymptomatic *A. hydrophila* infections in a cohort of Egyptian children, Abu Homos, 2004-2007

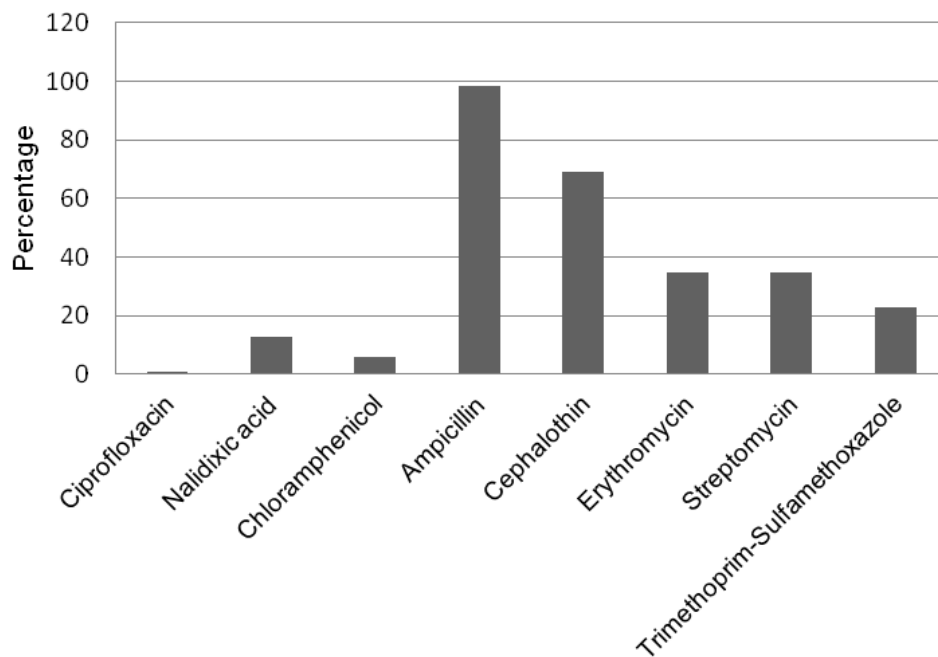


Table 1. Clinical characteristics of symptomatic *A. hydrophila* infections in a cohort of Egyptian children, Abu Homos, 2004-2007

Clinical characteristics of sole pathogen diarrhea cases (n = 20)	
Median age in months (interquartile range, (IQR))	8 (6.17)
Male, n (%)	13 (55)
Warm season, n (%)	17 (85)
Clinical features	
Fever, n (%)	11 (55)
Median days of illness (IQR)	4 (2.5)
Vomiting, n (%)	3 (15)
Maximum number of loose stool (IQR)	6 (4.8)

The incidence rate (IR) of isolating *A. hydrophila* as a sole pathogen from the stool of S children was 0.07 episode/child/year. In comparison, the incidence of ETEC as a sole pathogen in diarrheal samples was 1.26, *Campylobacter* was 0.47, *Shigella* was 0.06, and *Salmonella* 0.02 episode/child/year.

The peak incidence of *A. hydrophila*-associated diarrhea occurred in the second six months of life (IR 0.13 episode/child/year). Males had 62.5% (35/56) of the episodes. Episodes occurred mainly in the warm season, from May to October (18/20, 90%). The median duration of *A. hydrophila*-associated diarrhea was four days (interquartile range, IQR, 2-8 days) and the median maximum number of loose stools in any day during the episode was six (IQR 4-7).

Clinically, episodes of *A. hydrophila*-associated diarrhea were characterized by fever in 40% (8/20) and vomiting in 15% (3/20) of cases (Table). Of 34 episodes of diarrhea (any cause) associated with dehydration in the entire cohort (34/4001, 0.9%), only one episode was associated with *Aeromonas* infection (1/20, 5%), while 18 were associated with ETEC (18/636, 2.8%), 11 with rotavirus (11/153, 7%) and one case each with *Campylobacter* spp. (1/240, 0.4%) and *Shigella* spp. (1/29, 3.4%).

Among the children colonized with *A. hydrophila* in the cohort, five children had persistent shedding after the end of the diarrhea episode as defined by isolation of *A. hydrophila* from stool for at least two weeks of the follow-up period after the diarrhea episode ended. Shedding was not related to diarrhea and continued for two weeks as AS colonization in only one child. On the other hand, shedding preceded the diarrhea episode by three days in one child and by two weeks in another child.

Antibiotic susceptibility testing of all *A. hydrophila* isolates demonstrated the following

resistance pattern: cephalothin (69%), ciprofloxacin (1%) and chloramphenicol (6 %) (Figure 2).

Discussion

In a cohort of children living in a rural setting in the Nile River Delta of Egypt, we were able to demonstrate that *Aeromonas* spp was associated with diarrhea among study children. This is in agreement with previous reports where *Aeromonas* spp infection rates have ranged from 1.0% to 28% [8-10]. A unique feature of the current study is the population-based design with systematic collection of stools from children with and without diarrhea, allowing for a more precise description of *Aeromonas*-associated diarrhea. The pathogenic role of *A. hydrophila* as an enteric pathogen causing gastroenteritis is difficult to confirm because of the frequency of other pathogens isolated with *A. hydrophila* in symptomatic and asymptomatic subjects. However, we demonstrated that isolation of *A. hydrophila* as a sole pathogen was significantly more common in children presenting with diarrhea compared to children without diarrhea.

In the current study the isolation rate of *A. hydrophila* in symptomatic children (1.4%) was comparable to that of *Shigella* and was more common than *Salmonella*. This observation was similar to data obtained in cases with traveller's diarrhea in Europe, Asia and Africa [11]. Previous studies in Egypt showed that *Aeromonas* spp. are associated with diarrhea and in one study the *Aeromonas*-associated diarrhea was linked to local drinking water source [5, 6].

Although *A. hydrophila*-associated gastroenteritis can have a varied clinical presentation, in the present study, acute watery enteritis was the most common symptom. This observation is in agreement with those of previous studies [4,8,12]. In addition, the rate of

fever and vomiting among children with *Aeromonas*-associated diarrhea in the present study is similar to that seen in other reports [13,14].

Treatment of bacterial-associated diarrhea has become more complicated by the frequency of antimicrobial resistance in many pathogens [15]. Data regarding antimicrobial resistance for *A. hydrophila* have varied. We found relatively high resistance rates for cephalothin (69%) and trimethoprim-sulfamethoxazole (23%). In our study, we found low rates of resistance to ciprofloxacin and chloramphenicol. With high levels of resistance to many antibiotics, resistance of *A. hydrophila* to ciprofloxacin is still very low, which may suggest that ciprofloxacin and other quinolone class antimicrobials may be considered as potential drugs for the treatment of bacterial diarrhea caused by *A. hydrophila*.

Aeromonas spp. pathogenicity can be influenced by various virulence factors and also by the immune status of the infected child [16-18]. The role of *A. hydrophila* as a potential enteric pathogen in pediatric diarrhea, its associated virulence factors, and mechanisms of multi-drug resistance need further studies.

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